Studies of the Molecular Mechanism of the AmpC β-Lactamase from *Escherichia coli*

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Cephalosporinases related to the AmpC β-lactamase of *E. coli* contribute largely to resistance of Gram-negative organisms to modern β-lactam antibiotics.

Following solution of the crystal structure of the enzyme from *Citrobacter freundii* (Oeffner *et al.*, 1990) a number of site-directed mutations were designed to test the role of particular amino-acids in catalysis. These mutations were made in the homologous *E. coli* enzyme. The crystal structures of the wild-type and mutant enzymes reveal that the mutations have not produced large structural rearrangements. Nevertheless, steady-state kinetic measurements show sometimes very large effects of the mutations, which were not always those expected by inspection of the native enzyme. In part, this is due to the complicated kinetic mechanism of the β-lactamases. Kinetic and spectroscopic measurements provide ample evidence for there being at least two forms of the enzyme, which operate parallel, slowly interconverting catalytic cycles. Some of the mutations have an essential role in the reaction of one form of the enzyme but not the other. Further, it appears that different types of β-lactams utilize the substrate binding site in different ways in the two forms, so that the mutations can appear to have quite diverse effects. These properties of the enzymes complicate the interpretation of the effects of the mutations in terms of the known crystal structure.

Oeffner, C., D'Arcy, A., Daly, J.J., Gubernator, K., Charnas, R.L., Heinze, I., Hubschwerlen, C., & Winkler, F. (1990) Nature **343**, 284-288

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